SEPARATION OF INSECT AND PLANT MATERIAL FROM SCREEN-SWEEP SAMPLES[1]

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ABSTRACT: An alcohol technique which separates plant debris from insect material in screen-sweep samples is described. Data show this method can be up to 100% efficient at separating Chalcidoidea (Insecta: Hymenoptera) from bulk plant material when 95% ethanol is used. This technique opens up new habitats for collecting, decreases time needed to sort a sample, and reduces storage space required to house samples.

Screen-sweeping, a technique pioneered by Lubomir Masner, (personal communication), is an effective method for collecting large numbers of small insects (e.g., see Noyes 1989), and the use of a screen-sweep net is the first step in reducing plant debris in a sample. Triangular net heads are used when collecting to maximize the surface area of the net in contact with the ground. A ¼ inch galvanized hardware cloth screen fitted over the net opening effectively prevents many leaves, stems, stones, and twigs from entering the net bag (L. Masner, pers. comm., Noyes 1982, 1988). Polyethylene matting used in place of hardware cloth results in fewer damaged specimens (L. Masner, pers. comm.) but is less durable under rugged collecting conditions. In the field, the net bag is periodically emptied into gallon size heavy duty plastic freezer bags. The sample in the bag is sprayed with a saturated salt solution (NaCl in H_2O) which drowns and preserves small insects. We are concerned primarily with collecting the superfamily Chalcidoidea (Hymenoptera), parasitic wasps which are usually 1-5 mm long. Of special interest are Encyrtidae, many of which are found in dense grasses at ground level. However, even with a screen, sweeping such habitats quickly results in a ball of plant material inside the net which can damage delicate specimens. In addition, processing and subsequent laboratory sorting of such samples can be time-consuming. We describe here an alcohol flotation technique which further separates plant debris from insect material in a screen-sweep sample. This technique opens up new habitats for collection, decreases time needed to sort a sample, and reduces storage space required to house samples.

APPARATUS

The individual parts needed are easily constructed of Rubbermaid®

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Servin' Saver™ plastic containers. The main units employed by this method are a fine mesh strainer, a separation chamber, and a transport/pouring container (Fig. 1).

Fine mesh strainer (Fig. 1, A). The strainer is comprised of two tall 1.4 liter containers with their bottoms removed. The internal dimensions of each container are 12 x 12 x 13 cm. A suitable fine mesh material is stretched across the bottom of one container, which is then pushed tightly into the second container to form a taut screen. A small section of panty hose makes an excellent straining material, as it is strong, has an intricate weave which prevents even tiny insects from passing through, and insects or plant material do not cling to it.

Separation chamber (Fig. 1, B). The separation chamber is formed by a 2.4 and 4.5 liter square container. The bottom is removed from the smaller container and its sides are shortened so that it fits inside the larger container. The internal dimensions of the smaller container are 21 x 21 x 7.5 cm. A screen bottom made of ¼ inch mesh galvanized hardware cloth is attached near the bottom of the smaller container. Silicone rubber aquarium sealer can be used to affix the hardware cloth screen 1 cm above the bottom rim. The internal dimensions of the 4.5 liter container are 24.5 x 24.5 x 11 cm.

Figure 1. Apparatus used in separation procedure. From left to right are the fine mesh strainer (A), separation chamber (B), and transport/pouring container (C).

Transport/pouring container (Fig. 1, C). A tall 5 liter container measuring 20 x 20 x 22 cm is used for transportation in the field, storage, and as a receptacle when pouring alcohol.

Other equipment. Washing bottles are needed for rinsing specimens from the fine mesh strainer. Different sizes of funnels are used to facilitate the transfer of insects from the strainer into storage containers and for filtering dirty alcohol. An alcohol hydrometer and graduated cylinder are needed to monitor the concentration of alcohol used in the process.

PROCEDURE

Samples should be processed the day of collection to insure maximum efficiency of separation. The contents of a single freezer bag are transferred into the fine mesh strainer. This is easily done by cutting a bottom corner of the bag and pouring the sample into the strainer. If the bag is very full, the sample should be divided in half for processing. Once in the strainer, a gentle stream of water should be played over the sample for 3-4 minutes to flush out the brine. The sample is allowed to drain for a few minutes.

The sample is then transferred to the separation unit and enough 95% ethanol added to nearly fill the nested containers. Insects sink through the bottom screen of the inner container into the larger 4.5 liter container. Plant material such as flowers, leaves, seeds, and stems floats to the surface, or sinks and is restrained by the hardware cloth screen. To insure that the maximum number of insects sinks through the mesh, the sample is agitated for 4-5 minutes by gently shaking the inner container and stirring the debris. This breaks up any plant material packed together on the surface or obstructing the mesh bottom. The inner unit is then lifted out along with the bulk of the plant material, which can be discarded.

The alcohol and insects left in the 4.5 liter container are poured through the fine mesh strainer into the 5 liter container. The strainer traps the insects in the alcohol, and from there the sample is spooned or flushed with alcohol into a suitable container and stored in ethanol for later sorting in the laboratory. The alcohol in the storage containers should be changed after 24 hours. Insects stored in alcohol keep best if housed in a freezer (Masner and Goulet 1981).

Alcohol employed in the separation process which remains clean can be used again. To prevent the deposition of dirt onto specimens, dirty alcohol can be filtered and then reused for the next sample. A vacuum filtering flask is most effective for filtering in the laboratory. In the field, large cone-shaped paper coffee filters inserted into a wide funnel are

effective at removing dirt from alcohol. Paper coffee filters are thinner and work faster than standard laboratory filter paper.

FIELD TRIALS

Tests were conducted to determine the effectiveness of this technique. Screen-sweep samples were collected and subjected to the separation process. Floating plant portions were retained and examined to ascertain the number of insects that would potentially be lost with this technique. To save time, we only sorted to certain categories of Hymenoptera (Tables 1 and 2). Of those categories, "Other Chalcidoidea" includes families such as Eulophidae, Eurytomidae, Pteromalidae, and Torymidae. "Other Microhymenoptera" are small non-chalcidoid wasps such as Cynipoidea, Proctotrupoidea, and Scelionoidea. The first three tests used the same 70% ethanol (Table 1).

Specimens which sank would have been retained while those which floated with the plant material would have been discarded. In categories with large sample sizes the percentage of chalcidoid specimens which floated and would have been discarded ranged from 0.7-5.6 percent (Table 1).

In additional tests using the same alcohol, the loss rate reached 10-18% for some categories of Hymenoptera. We hypothesized that the alcohol used in the separation process was becoming diluted with water and allowing more insects to float. In addition to any moisture inherent in the samples, they were being subjected to the brine and a water rinse, all of which could introduce water into the alcohol. To test this hypothesis, two of us swept a local grassy meadow for one hour each. This collecting site is characterized by having a rich chalcidoid fauna in very dense, mature grasses. The total weight of the samples collected was 1.14 kg. The samples were combined and divided into six equal portions based on weight and subjected to the separation process using 50, 70, and 95% ethanol with two repetitions, each using fresh alcohol. The percentage of the total number of insects from each category that would have been discarded is shown in Table 2.

The test using 50% ethanol gave poor results, with losses often exceeding 20% of the total collected. The test with 70% ethanol reflected our earlier findings, with chalcidoid losses ranging from about 1-7% for categories with large sample sizes. The test with 95% ethanol produced the best results, and insect loss was from 0 - 2.8% for all but one category of Hymenoptera (Table 2).

Although alcohol used in the separation process will become diluted, using an alcohol hydrometer to monitor concentration will prevent the

undue loss of valuable specimens. Based on 21 freezer bag samples, each bag processed decreases alcohol concentration by approximately 4 percent. We discard our alcohol when the concentration reaches 70-75%, the level where our data show we may begin to lose 5% of the specimens.

Table 1. Results using the same 70% ethanol for consecutive tests in the separation process. Data are expressed as % (N) where % is the percentage of total insects (N) from each category which would have been discarded with the plant material. Specimens collected from Texas, Jim Wells Co., La Copita Research Station, 20.V.1987.

	Test 1	Test 2	Test 3
Aphelinidae & Trichogrammatidae	0.0 (89)	1.8 (108)	0.7 (409)
Encyrtidae	5.6 (125)	4.6 (108)	3.7 (463)
Eucharitidae	0.0 (1)	25.0 (4)	18.7 (16)
Eupelmidae	0.0 (3)	0.0 (1)	0.0 (7)
Mymaridae	1.3 (223)	4.5 (111)	1.0 (380)
Other Chalcidoidea	5.6 (531)	2.3 (683)	5.5 (2023)
Other Microhymenoptera	14.7 (68)	14.0 (71)	14.0 (410)
Ichneumonoidea	3.3 (30)	4.0 (94)	8.6 (29)
Aculeates	75.0 (4)	12.5 (8)	24.4 (41)

Table 2. Percentage of the total number of insects from each category which would have been discarded in two repetitions using fresh 50, 70, and 95% ethanol in the separation process. Data are expressed as % (N) where % is the percentage of total insects (N) from each category. Specimens collected from Texas, Brazos Co., Lick Creek Park, 4.VIII.1987.

	50% Ethanol		70% Ethanol		95% Ethanol	
	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
Aphelinidae & Trichogrammatidae	26.7 (45)	9.3 (172)	3.1 (159)	2.2 (92)	0.0 (106)	2.8 (143)
Encyrtidae	22.4 (85)	6.5 (138)	4.6 (132)	1.3 (76)	0.0 (128)	2.6 (117)
Eupelmidae	0.0 (6)	0.0 (6)	7.7 (13)	25.0 (4)	0.0 (6)	0.0 (12)
Mymaridae	23.4 (77)	5.4 (167)	6.6 (211)	5.4 (110)	0.7 (136)	1.4 (146)
Other Chalcidoidea	21.5 (195)	6.3 (319)	2.5 (318)	3.2 (190)	0.4 (260)	0.0 (244)
Other Microhymenoptera	41.0 (22)	11.9 (42)	2.9 (34)	22.0 (18)	0.0 (28)	13.3 (15)
Ichneumonoidea	25.0 (4)	15.4 (13)	18.8 (16)	9.1 (11)	0.0 (14)	0.0 (2)
Aculeates	0.0 (1)	—	—	—	0.0 (4)	0.0 (5)

SUMMARY

There are many advantages of this plant separation technique. The required parts are inexpensive, easy to assemble, and readily transportable in the field. We have employed this technique in campgrounds, motels, and the laboratory. The effective removal of extraneous plant material from screen-sweep samples dramatically decreases the time and space required to sort and house them. Although this technique has been shown to be effective for small Hymenoptera, it is hoped that other collectors will utilize it. Using this method, we commonly collect many Collembola, Microcoryphia, Thysanoptera, Hemiptera, Homoptera, Coleoptera, Diptera, and Arachnida. The use of this technique may encourage workers to sample habitats which in the past were unpalatable.

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